A

Practitioner's Docket No. MSU 4.1-406 ***PATENT***

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of

Inventor(s): Alberto Leonel Mendoza

WARNING: *37 C.F.R. § 1.41(a)(1) points out:*

"(a) A patent is applied for in the name or names of the actual inventor or inventors.

"(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.63, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors."

For (title): METHOD AND VACCINE FOR TREATMENT OF PYTHIOSIS INSIDIOSI IN HUMANS AND LOWER ANIMALS

CERTIFICATION UNDER 37 C.F.R. 1.10*
(Express Mail label number is mandatory.)
(Express Mail certification is optional.)

I hereby certify that this New Application Transmittal and the documents referred to as attached therein are being deposited with the United States Postal Service on this date May 20, 1998, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number EI958529238US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Tammi L. Taylor
(type or print name of person mailing paper)

Tammi L. Taylor
Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

***WARNING:** Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

1. Type of Application

This new application is for a(n)

(check one applicable item below)

☐ Original (nonprovisional)

☐ Design

☐ Plant

WARNING: ***Do not*** *use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4), unless the International Application is being filed as a divisional, continuation or continuation-in-part application.*

WARNING: ***Do not*** *use this transmittal for the filing of a provisional application.*

NOTE: If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

☒ Divisional.

☐ Continuation.

☐ Continuation-in-part (C-I-P).

2. Benefit of Prior U.S. Application(s) (35 U.S.C. 119(e), 120, or 121)

NOTE: A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copending nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U.S.C. 112. Each prior application must also be:

(i) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America; or

(ii) Complete as set forth in § 1.51(b); or

(iii) Entitled to a filing date as set forth in § 1.53(b) or § 1.53(d) and include the basic filing fee set forth in § 1.16; or

(iv) Entitled to a filing date as set forth in § 1.53(b) and have paid therein the processing and retention fee set forth in § 1.21(l) within the time period set forth in § 1.53(f).

37 C.F.R. § 1.78(a)(1).

NOTE: If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

WARNING: *When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application* ***must*** *be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3).*

☒ The new application being transmitted claims the benefit of prior U.S. application(s). Enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. Papers Enclosed

A. Required for filing date under 37 C.F.R. § 1.53(b) (Regular) or 37 C.F.R. § 1.153 (Design) Application

17 Pages of specification

6 Pages of claims

0 Sheets of drawing

☐ formal

☐ informal

B. Other Papers Enclosed

1 Pages of Abstract

_____ Other

WARNING: ***DO NOT*** *submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. For comments on proposed then-new 37 CFR 1.84, see Notice of March 9, 1988 (1990 O.G. 57-62).*

NOTE: "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (5/8 inch) down from the top of the page . . ." 37 C.F.R. 1.84(c)).

(complete the following, if applicable)

☐ The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)." 37 C.F.R. 1.84(b).

4. Additional papers enclosed

☒ Preliminary Amendment

☒ Information Disclosure Statement (37 C.F.R. 1.98)

☒ Form PTO–1449 (PTO/SB/08A and 08B)

☒ Citations

☐ Declaration of Biological Deposit

☐ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.

☐ Authorization of Attorney(s) to Accept and Follow Instructions from Representative

☐ Special Comments

☐ Other

5. Declaration or oath

NOTE: A newly executed declaration is not required in a continuation or divisional application provided that the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. The copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under § 1.47, then a copy of that declaration must be filed accompanied by a copy of the decision granting § 1.47 status or, if a nonsigning person under § 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed. See 37 C.F.R. §§ 1.63(d).

☒ Enclosed

Executed by

(check **all** *applicable boxes)*

☒ inventor(s). As filed in parent Application Serial No. 08/895,940 Filed 07/17/97

☐ legal representative of inventor(s). 37 CFR 1.42 or 1.43.

☐ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.

☐ This is the petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 is also attached. See item 13 below for fee.

☐ Not Enclosed.

NOTE: Where the filing is a completion in the U.S. of an International Application or where the completion of the U.S. application contains subject matter in addition to the International Application, the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.

☐ Application is made by a person authorized under 37 C.F.R. 1.41(c) on behalf of *all* the above named inventor(s).

(The declaration or oath, along with the surcharge required by 37 CFR 1.16(e) can be filed subsequently).

NOTE: It is important that all the correct inventor(s) are named for filing under 37 CFR 1.41(c) and 1.53(b).

☐ Showing that the filing is authorized.
(not required unless called into question. 37 CFR 1.41(d))

6. Inventorship Statement

WARNING: *If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.*

The inventorship for all the claims in this application are:

☒ The same.

or

☐ Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,

☐ is submitted.

☐ will be submitted.

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. An English translation of the non-English language application and the processing fee of $130.00 required by 37 CFR 1.17(k) is required to be filed with the application, or within such time as may be set by the Office. 37 CFR 1.52(d).

☒ English

☐ Non-English

☐ The attached translation includes a statement that the translation is accurate. 37 C.F.R. 1.52(d).

8. Assignment

☒ An assignment of the invention to Board of Trustees operating Michigan State University 412 Administration Bldg.-MSU, East Lansing MI 48824

☐ is attached. A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached. Was filed and recorded in Parent Appln. Serial No. 08/895,940 filed 07/17/97.

☐ will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters-one for the application and one for the assignment." Notice of May 4, 1990 (1114 O.G. 77-78).

***WARNING:** A newly executed "CERTIFICATE UNDER 37 CFR 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993, 1150 O.G. 62-64.*

9. Certified Copy

Certified copy(ies) of application(s)

Country	Appln. No.	Filed
Country	Appln. No.	Filed
Country	Appln. No.	Filed

from which priority is claimed

☐ is (are) attached.

☐ will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration. 37 CFR 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application, then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 C.F.R. 1.16)

A. ☒ Regular application

CLAIMS AS FILED				
Number filed	Number Extra		Rate	Basic Fee 37 C.F.R. 1.16(a) $790.00
Total Claims (37 CFR 1.16(c)) 10 − 20 =	−0−	×	$ 22.00	−0−
Independent Claims (37 CFR 1.16(b)) 2 − 3 =	−0−	×	$ 82.00	−0−
Multiple dependent claim(s), if any (37 CFR 1.16(d))	1	+	$270.00	270.00

☒ Amendment cancelling extra claims is enclosed.

☐ Amendment deleting multiple-dependencies is enclosed.

☐ Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 CFR 1.16(d).

Filing Fee Calculation $1,060.00

B. ☐ Design application ($330.00—37 CFR 1.16(f))

Filing Fee Calculation $_____

C. ☐ Plant application ($540.00—37 CFR 1.16(g))

Filing fee calculation $_____

11. Small Entity Statement(s)

☐ Statement(s) that this is a filing by a small entity under 37 CFR 1.9 and 1.27 is (are) attached.

WARNING: *"Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent, including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under § 1.53 as a continuation, division, or continuation-in-part (including a continued prosecution application under § 1.53(d)), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) of a prior application, or a reissue application may rely on a statement filed in the prior application or in the patent if the nonprovisional application or the reissue application includes a reference to the statement in the prior application or in the patent or includes a copy of the statement in the prior application or in the patent and status as a small entity is still proper and desired. The payment of the small entity basic statutory filing fee will be treated as such a reference for purposes of this section." 37 C.F.R. § 1.28(a)(2).*

(complete the following, if applicable)

☒ Status as a small entity was claimed in prior application 08 / 895,940, filed on 07/17/98, from which benefit is being claimed for this application under:

35 U.S.C. ☐ 119(e),
☐ 120,
☒ 121,
☐ 365(c),

and which status as a small entity is still proper and desired.

☒ A copy of the statement in the prior application is included.

Filing Fee Calculation (50% of **A, B** or **C** above)

$530.00

NOTE: Any excess of the full fee paid will be refunded if small entitiy status is established and a refund request are filed within 2 months of the date of timely payment of a full fee. The two-month period is not extendable under § 1.136. 37 CFR 1.28(a).

12. Request for International-Type Search (37 C.F.R. 1.104(d))

(complete, if applicable)

☐ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made at This Time

☐ Not Enclosed

☐ No filing fee is to be paid at this time.
(This and the surcharge required by 37 C.F.R. 1.16(e) can be paid subsequently.)

☒ Enclosed

☒ Filing fee	$ 530.00
☐ Recording assignment ($40.00; 37 C.F.R. 1.21(h)) (See attached "COVER SHEET FOR ASSIGNMENT ACCOMPANYING NEW APPLICATION".)	$ _____
☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached ($130.00; 37 C.F.R. 1.47 and 1.17(i))	$ _____
☐ For processing an application with a specification in a non-English language ($130.00; 37 C.F.R. 1.52(d) and 1.17(k))	$ _____
☐ Processing and retention fee ($130.00; 37 C.F.R. 1.53(d) and 1.21(l))	$ _____
☐ Fee for international-type search report ($40.00; 37 C.F.R. 1.21(e))	$ _____

NOTE: 37 CFR 1.21(l) establishes a fee for processing and retaining any application that is abandoned for failing to complete the application pursuant to 37 CFR 1.53(f) and this, as well as the changes to 37 CFR 1.53 and 1.78(a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid, or the processing and retention fee of § 1.21(l) must be paid, within 1 year from notification under § 53(f).

Total fees enclosed $530.00

14. Method of Payment of Fees

☒ Check in the amount of $530.00

☐ Charge Account No. _____ in the amount of $_____.

A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 CFR 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: *If no fees are to be paid on filing, the following items should not be completed.*

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.*

☒ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No. 13-0610:

☒ 37 C.F.R. 1.16(a), (f) or (g) (filing fees)

☒ 37 C.F.R. 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

☐ 37 C.F.R. 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

☒ 37 C.F.R. §§ 1.17(a)(1)–(5) (extension fees pursuant to § 1.136(a)).

☒ 37 C.F.R. 1.17 (application processing fees)

NOTE: ". . .A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

☐ 37 C.F.R. 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1.311(b).

NOTE: 37 CFR 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying, . . . issue fee." From the wording of 37 CFR 1.28(b), (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions as to Overpayment

NOTE: ". . . Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

☒ Credit Account No. 13-0610

☐ Refund

SIGNATURE OF PRACTITIONER

Reg. No. 20,931

Ian C. McLeod

(type or print name of attorney)

Tel. No. (517) 347-4100

2190 Commons Parkway

P.O. Address

Customer No. 21036

Okemos, Michigan 48864

☒ **Incorporation by reference of added pages**

(check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

☒ Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added 5

☐ Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added _____

☐ Plus added pages deleting names of inventor(s) named in prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application.

Number of pages added _____

☐ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added _____

☐ **Statement Where No Further Pages Added**

(if no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item)

☐ This transmittal ends with this page.

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ADDED PAGES FOR APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED

NOTE: See 37 CFR 1.78.

17. Relate Back

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

(complete the following, if applicable)

☒ Amend the specification by inserting, before the first line, the following sentence:

A. 35 U.S.C. 119(e)

NOTE: "Any nonprovisional application claiming the benefit of one or more prior filed copending provisional applications must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior provisional application, identifying it as a provisional application, and including the provisional application number (consisting of series code and serial number)." 37 C.F.R. § 1.78(a)(4).

☐ "This application claims the benefit of U.S. Provisional Application(s) No(s).:

APPLICATION NO(S).:	**FILING DATE**
_____ /_____	_____ "
_____ /_____	_____ "
_____ /_____	_____ "

B. 35 U.S.C. 120, 121 and 365(c)

NOTE: "Except for a continued prosecution application filed under § 1.53(d), any nonprovisional application claiming the benefit of one or more prior filed copending nonprovisional applications or international applications designating the United States of America must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior application, identifying it by application number (consisting of the series code and serial number) or international application number and international filing date and indicating the relationship of the applications. . . . Cross-references to other related applications may be made when appropriate." (See § 1.14(a)). 37 C.F.R. § 1.78(a)(2).

☒ "This application is a

- ☐ continuation
- ☐ continuation-in-part
- ☒ divisional

of copending application(s)

☒ application number 08/ 895,940 filed on 07/17/97 "

☐ International Application _____ filed on _____ and which designated the U.S."

NOTE: The proper reference to a prior filed PCT application that entered the U.S. national phase is the U.S. serial number and the filing date of the PCT application that designated the U.S.

NOTE: (1) Where the application being transmitted adds subject matter to the International Application, then the filing can be as a continuation-in-part or (2) if it is desired to do so for other reasons then the filing can be as a continuation.

NOTE: The deadline for entering the national phase in the U.S. for an international application was clarified in the Notice of April 28, 1987 (1079 O.G. 32 to 46) as follows:

"The Patent and Trademark Office considers the International application to be pending until the 22nd month from the priority date if the United States has been designated and no Demand for International Preliminary Examination has been filed prior to the expiration of the 19th month from the priority date and until the 32nd month from the priority date if a Demand for International Preliminary Examination which elected the United States of America has been filed prior to the expiration of the 19th month from the priority date, provided that a copy of the international application has been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively. If a copy of the international application has not been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively, the international application becomes abandoned as to the United States 20 or 30 months from the priority date respectivley. These periods have been placed in the rules as paragraph (h) of § 1.494 and paragraph (i) of § 1.495. A continuing application under 35 U.S.C. 365(c) and 120 may be filed anytime during the pendency of the international application."

☐ "The nonprovisional application designated above, namely application _____ / _____, filed _____, claims the benefit of U.S. Provisional Application(s) No(s).:

APPLICATION NO(S).:	FILING DATE
_____ / _____	_____ "
_____ / _____	_____ "
_____ / _____	_____ "

☐ Where more than one reference is made above, please combine all references into one sentence.

18. Relate Back—35 U.S.C. 119 Priority Claim for Prior Application

The prior U.S. application(s), including any prior International Application designating the U.S., identified above in item 17B, in turn itself claim(s) foreign priority(ies) as follows:

Country	Appln. no.	Filed on

The certified copy(ies) has (have)

☐ been filed on _____, in prior application 0 /_____, which was filed on _____.

☐ is (are) attached.

WARNING: *The certified copy of the priority application that may have been communicated to the PTO by the International Bureau may **not** be relied on without any need to file a certified copy of the priority application **in the continuing application.** This is so because the certified copy of the priority application communicated by the International Bureau is placed in a folder and is not assigned a U.S. serial number unless the national stage is entered. Such folders are disposed of if the national stage is not entered. Therefore, such certified copies may not be available if needed later in the prosecution of a continuing application. An alternative would be to physically remove the priority documents from the folders and transfer them to the continuing application. The resources required to request transfer, retrieve the folders, make suitable record notations, transfer the certified copies, enter and make a record of such copies in the Continuing Application are substantial. Accordingly, the priority documents in folders of international applications that have not entered the national stage may not be relied on. Notice of April 28, 1987 (1079 O.G. 32 to 46).*

19. Maintenance of Copendency of Prior Application

NOTE: The PTO finds it useful if a copy of the petition filed in the prior application extending the term for response is filed with the papers constituting the filing of the continuation application. Notice of November 5, 1985 (1060 O.G. 27).

A. ☐ Extension of time in prior application

*(This item **must** be completed and the papers filed **in the prior application,** if the period set in the prior application has run.)*

☐ A petition, fee and response extends the term in the pending **prior** application until _____.

☐ A **copy** of the petition filed in prior application is attached.

B. ☐ Conditional Petition for Extension of Time in Prior Application

(complete this item, if previous item not applicable)

☐ A conditional petition for extension of time is being filed in the pending **prior** application.

☐ A **copy** of the conditional petition filed in the prior application is attached.

20. Further Inventorship Statement Where Benefit of Prior Application(s) Claimed

(complete applicable item (a), (b) and/or (c) below)

(a) ☒ This application discloses and claims only subject matter disclosed in the prior application whose particulars are set out above and the inventor(s) in this application are

- ☒ the same.
- ☐ less than those named in the prior application. It is requested that the following inventor(s) identified for the prior application be deleted:

_____

(type name(s) of inventor(s) to be deleted)

(b) ☐ This application discloses and claims additional disclosure by amendment and a new declaration or oath is being filed. With respect to the prior application, the inventor(s) in this application are

- ☐ the same.
- ☐ the following additional inventor(s) have been added:

_____

(type name(s) of inventor(s) to be added)

(c) The inventorship for all the claims in this application are

- ☒ the same.
- ☐ not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made
  - ☐ is submitted.
  - ☐ will be submitted.

21. Abandonment of Prior Application *(if applicable)*

☐ Please abandon the prior application at a time while the prior application is pending, or when the petition for extension of time or to revive in that application is granted, and when this application is granted a filing date, so as to make this application copending with said prior application.

NOTE: According to the Notice of May 13, 1983 (103, TMOG 6-7), the filing of a continuation or continuation-in-part application is a proper response with respect to a petition for extension of time or a petition to revive and should include the express abandonment of the prior application conditioned upon the granting of the petition and the granting of a filing date to the continuing application.

22. Petition for Suspension of Prosecution for the Time Necessary to File an Amendment

WARNING: *"The claims of a new application may be finally rejected in the first Office action in those situations where (1) the new application is a continuing application of, or a substitute for, an earlier application, and (2) all the claims of the new application (a) are drawn to the same invention claimed in the earlier application, and (b) would have been properly finally rejected on the grounds of art of record in the next Office action if they had been entered in the earlier application." MPEP, § 706.07(b).*

NOTE: Where it is possible that the claims on file will give rise to a first action final for this continuation application and for some reason an amendment cannot be filed promptly (e.g., experimental data is being gathered) it may be desirable to file a petition for suspension of prosecution for the time necessary.

(check the next item, if applicable)

☐ There is provided herewith a Petition To Suspend Prosecution for the Time Necessary to File An Amendment (New Application Filed Concurrently)

23. Small Entity (37 CFR § 1.28(a))

☒ Applicant has established small entity status by the filing of a statement in parent application 08/895,940 on 07/17/97

☒ A copy of the statement previously filed is included.

WARNING: *See 37 CFR § 1.28(a).*

24. NOTIFICATION IN PARENT APPLICATION OF THIS FILING

☐ A notification of the filing of this
(check one of the following)

- ☐ continuation
- ☐ continuation-in-part
- ☐ divisional

is being filed in the parent application, from which this application claims priority under 35 U.S.C. § 120.

MSU 4.1-406
5/18/98

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Albert Leonel Mendoza

For : METHOD AND VACCINE FOR TREATMENT OF PYTHIOSIS INSIDIOSI IN HUMANS AND LOWER ANIMALS

Assistant Commissioner For Patents

Washington, D. C. 20231

AMENDMENT UNDER 37 CFR 1.111

Sir:

Preliminary to the first Office Action, Applicant amends and remarks as follows.

In The Claims

Cancel Claims 1-15.

REMARKS

An Office Action on the merits is requested.

Respectfully,

Ian C. McLeod
Registration No. 20,931

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Attorney's Docket No. MSU 4.1-356 ***PATENT***

☒ Applicant Alberto Leonel Mendoza ☐ Patentee _____

☐ Application No. _____ ☐ Patent No. _____

☐ Filed on _____ ☐ Issued on _____

Title: METHOD AND VACCINE FOR TREATMENT OF PYTHIOSIS INSIDIOSI IN HUMANS AND LOWER ANIMALS

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(d))—NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

Name of Nonprofit Organization MICHIGAN STATE UNIVERSITY

Address of Nonprofit Organization 238 Administration Bldg.

East Lansing, Michigan 48824

TYPE OF NONPROFIT ORGANIZATION

☒ University or Other Institution of Higher Education

☐ Tax Exempt Under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3))

☐ Nonprofit Scientific or Educational Under Statute of State of the United States of America

(Name of State _____)

(Citation of Statute _____)

☐ Would Qualify as Tax Exempt Under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3)), if Located in the United States of America

☐ Would Qualify as Nonprofit Scientific or Educational Under Statute of State of the United States of America if Located in the United States of America

(Name of State _____)

(Citation of Statute _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization, as defined in 37 CFR 1.9(e), for purposes of paying reduced fees to the United States Patent and Trademark Office under Sections 41(a) and (b) of Title 35, United States Code, with regard to the invention described in

☒ the specification filed herewith, with title as listed above.

☐ the application identified above.

☐ the patent identified above.

I hereby declare that rights under contract or law have been conveyed to, and remain with, the nonprofit organization, with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 C.F.R. 1.9(c), if that person made the invention, or by any concern that would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e)

*NOTE: *Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27).*

Each such person, concern or organization having any rights in the invention is listed below:

☒ No such person, concern, or organization exists.

☐ Each such person, concern or organization is listed below.

Name _____

Address _____

_____

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

Name _____

Address _____

_____

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any charge in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing Stephen H. Terry

Title in Organization Michigan State University

Address of Person Signing 412 Administration BUilding

East Lansing, MI 48824

SIGNATURE _____ Date July 11, 1997

MSU 4.1-356
7/15/97

METHOD AND VACCINE FOR TREATMENT OF PYTHIOSIS INSIDIOSI IN HUMANS AND LOWER ANIMALS

BACKGROUND OF THE INVENTION

(1) Summary of the Invention

The present invention relates to protein vaccines and methods of use thereof for the treatment of *Pythium insidiosum* infections in humans and lower mammals. Further, the present invention relates to a method for preparing the preferred vaccine for the treatment which contains intracellular and extracellular proteins of *Pythium insidiosum*.

(2) Description of Related Art

Infections caused by fungal and parafungal organisms are occurring with increasing frequency in patients with debilitating illnesses such as leukemia and AIDS, as well as those undergoing immunosuppressive therapy. Within this group of organisms are the traditional pathogenic fungi and a long list of newly recognized emerging opportunistic fungal and parafungal organisms. Among the emerging pathogens is the oomycete *Pythium insidiosum* a fungal-like organism in the Kingdom Kromista, Phylum Pseudofungi. *Pythium insidiosum* is not only psychologically distinct from members of the Kingdom Fungi, but also differs physiologically. This may explain why anti-fungal drugs do not have any effect on pythiosis.

Pythiosis insidiosi particularly occurs in humans and lower animals in the tropical, subtropical, and temperate areas of the world (Cock, W.A.W., et al., J. Clin. Microbiol. **25**:344-349 (1987)). The disease was described in the beginning of the century in equines of tropical and subtropical countries including India and Indonesia as well as the USA. Soon, however, it was

evident that the disease not only affected equines but other mammalian species. In lower animals infections of the cutaneous tissues, lymphatic vessels, intestines, lungs, and bones have been found. In humans, a deadly arteritis infection, subcutaneous invasion and keratitis occurs.

The currently available drugs used to treat fungal infections have had little or no effect on *Pythium insidiosum*. Reports of treatment with either amphotericin B or surgery, commonly used to treat this disease in both humans and lower animals, have indicated that 60% of the patients died of their infections. In cases of arterial invasion in humans, amphotericin B did not eliminate the infection (Rinaldi, M.G., et al., Mycology Observer 9:7 (1989); and Thianprasit, M., Trop Dermathol **4**:1-4 (1990)), whereas in surgery the main problem has been to determine how much of the infected tissues has to be removed. Thus, relapses are common in surgically treated patients, who must also endure the pain and distress that such an invasive traumatic procedure inflicts on them.

The curative properties of *P. insidiosum* possessed curative properties was first noticed when Costarrican equine with pythiosis injected with *P. insidiosum* antigens, in a skin test, resulted in the cure of some of the horses (Mendoza, L., et al., Equine pythiosis in Costa Rica: report of 39 cases. Mycopathologia **94**:123-126 (1986)). Simultaneously, a similar vaccine with curative properties was successfully used in equines with the disease in Australia (Miller, R. I., Aust. Vet. J. **57**:377-382 (1981)). These two vaccines have been referred to in the literature as Mendoza's and Miller's vaccines respectively (Newton, J. C., et al., The Compendium **15**:491-493 (1993)). Early reports indicated that the antigens used in the *P. insidiosum*-vaccine possessed unique characteristics, somewhat similar to the features

of those reported in *Trichophyton verrucosum* (Gudding R., et al., Can. Vet. J. **36**:302-306 (1994)) and other immunotherapeutic vaccines (Foster, J. S., et al., Vet. Med. Small Ani. Clin. **71**, 920 (1976); Pier, A. C., et al., Equine Practice **15**:23-27 (1993)).

Miller's vaccine uses sonicated hyphal antigens (Miller, R. E., Aust. Vet. J. **57**:377-382 (1981)), while Mendoza's vaccine is prepared from culture filtrate antigens (Mendoza, L., et al., **94**:123-126 (1986)). Both vaccines have cured about 53% of vaccinated horses. Mendoza's vaccine, however, has a longer shelf life and milder side effects (Miller, R. I., et al., J. Am. Vet. Med. Assoc. **182**:1227-1229 (1983)). In addition to its immunotherapeutic features Mendoza's vaccine also showed some degree of protection. This protection was later found to be of short duration (Mendoza, L., et al., Mycopathologia **119**:89-95 (1992)). In 15 years of use more than 300 equines have been cured. Mendoza's vaccine was proved to be consistent and safe. In spite of this, the vaccine only cured early equine pythiosis, but not chronic cases of this disease (Mendoza, L., et al., Mycopathologia **119**:89-95 (1992)). Aside from the fact that the vaccine only cured early equine pythiosis cases, nothing was known about the immunogens involved in its curative properties nor the immune mechanisms that triggered the killing of *P. insidiosum's* hyphae infected tissues.

In a recent study using SDS-PAGE and Western blot analysis, the presence of three immunodominant hyphal proteins was found to be of interest (Mendoza, L., et al., J. Clin. Microbiol. **30**:2980-2983 (1992)). The immunoblotting study revealed that the IgG of sera from horses with active pythiosis recognized most of the proteins of *P. insidiosum*. However, of all the proteins analyzed, three bands, the 32,000-molecular-weight 32K, 30K, and 28K, were particularly prominent. More significantly was the finding that antibodies against

these three proteins persisted for long periods of time in the successfully vaccinated horses.

There is a need for vaccines which cure pythiosis. The need is particularly great where the patient is in the chronic stage of the disease.

OBJECTS

It is therefore an object of the present invention to provide a method for treating pythiosis in humans and lower animals. Further, it is an object of the present invention to provide vaccine compositions and methods for the preparation thereof. Further still, it is an object of the present invention to provide a method for curing pythiosis which is economical, reliable and effective. These and other objects will become increasingly apparent by reference to the following description.

DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention relates to an injectable vaccine for treatment of pythiosis which comprises in a sterile aqueous solution an admixture of: (a) intracellular proteins separated from disrupted cells of *Pythium insidiosum;* and (b) extracellular proteins from a supernatant from growing the cells of the *Pythium insidiosum*.

Further, the present invention relates to a method for providing an injectable vaccine for treatment of Pythiosis which comprises: (a) growing cells of *Pythium insidiosum* in a culture medium; (b) separating the cells from a first supernatant of the culture medium which contains extracellular proteins; (c) killing the cells; (d) disrupting the cells in sterile water; (e) separating the disrupted cells from the water to produce a second supernatant containing intracellular proteins; (f) mixing the first supernatant of step (b) with the second supernatant of step (e); (g) separating the combined proteins from the mixture of step (f); (h) mixing the separated proteins in sterile distilled

water; and (i) dialyzing the mixture of step (h) to remove low molecular weight components less than 10,000 MW to produce the vaccine.

Further, the present invention relates to a method for the treatment of Pythiosis in a mammal having the disease which comprises: (a) providing an injectable vaccine which comprises in a sterile aqueous solution in admixture: (1) an intracellular proteins separated from disrupted cells of *Pythium insidiosum*; and (2) extracellular proteins from a supernatant from growing the cells of the *Pythium insidiosum*; and (b) vaccinating the mammal with the vaccine.

Finally, the present invention relates to a method for treatment of pythiosis in human patients having the disease which comprises: (a) providing a vaccine containing separated proteins of *Pythium insidiosum* in a sterile aqueous solution; and (b) vaccinating the patient with the vaccine.

The *Pythium insidiosum* was deposited with the American Type Culture Collection under the Budapest Treaty as ATCC 58643. It is available upon request by name and number. All restrictions on distribution of ATCC 58643 are irrevocably removed on granting of a patent on this application. The address of the American Type Culture Collection is 12301 Parklawn Drive, Rockville, Maryland 20852.

Preferably the vaccine contains between about 3.0 and 2.0 mg of protein per ml. The vaccination dosage is between about 1.0 and 2.0 mg per kg of body weight of the mammal.

The vaccine of the present invention is preferably injected intramuscularly. The vaccine can also be administered intradermally or subcutaneously.

A sterile carrier or adjuvant is used in the vaccine. The preferred carrier is water or an aqueous saline solution, particularly in humans. An adjuvant for the vaccine is EMULSIGEN (MVP Labs, Ralston,

Nebraska), which is a paraffin oil in a water emulsion, which can be used in food animals. Freund's Incomplete Adjuvant, which is 15 percent by weight mannide monooleate and 85% paraffin oil, available from Difco, Detroit, Michigan, can be used in non-food (i.e. laboratory animals). The adjuvants aid in slowly releasing the vaccine into the animal and can potentiate the immune response. Any commercial oil emulsion adjuvants can be used such as vitamin E.

The vaccine can be combined with non-immunizing components for other diseases to produce a multivalent vaccine or with other medicaments, particularly antibiotics. The antibiotics can be used prior to vaccination.

In the following Example 1, the improved vaccine was prepared by adding cytoplasmic antigens to the earlier *P. insidiosum*-vaccine (Mendoza et al., Mycopathologica **119**:89-95 (1992)). In Example 2, the modified vaccine of Example 1 was tested in horses with chronic pythiosis insidiosi, only 48% of the horses were cured. All horses with acute pythiosis insidiosi were cured with this new vaccine. One advantage of the new vaccine is that the earlier vaccine always failed in chronic cases. Example 3 shows preparation of the proteins by recombinant methods. In Example 4, the modified vaccine was successfully tested in a Thai boy with pythiosis insidiosi. This Thai patient was diagnosed with an infection caused by *P. insidiosum* in his external carotid artery. In spite of efforts to treat the infection with traditional methods the patient did not show improvement. As a last resort *Pythium insidiosum* modified vaccine was given to him. The patient has been declared clinically cured.

EXAMPLE 1

1. *Pythium insidiosum* strain ATCC 58643, was transferred to a 1.0-liter flask containing 500 ml of Sabouraud dextrose broth (Difco, Detroit, MI).

2. Cultures were incubated at 37°C for five days on shaker rotating at 150 rpm.

3. Cultures were killed with Merthiolet (thimersol) (0.02% wt/vol), filtered to separate the cells (hyphae) from the liquid phase containing exoantigens of *P. insidiosum* (save the liquid phase in a sterile container to be used in step 6).

4. The cell mass obtained in step 3, was washed twice with sterile distilled water disrupted by sonication until 100% of the hyphae were fragmented. Other methods could be used such as a French press.

5. The mixture, obtained in step 4, was centrifuged at 5,000 x g for 20 minutes.

6. The supernatant was separated from the pellet (pellet can be eliminated) and then the supernatant was added to the liquid phase in step 3.

7. To confirm the presence of the immunodominant proteins in the supernatant obtained on step 6, the sample was subjected to SDS-PAGE electrophoresis and Western blot analysis as per Mendoza et al (J. Clin. Microbiol. **30**:2980-29-83 (1992)). Following electrophoresis, the prominent proteins were cut from the acrylamide gels and purified. A mixture of the three proteins were added to Mendoza's original vaccine (~2.0 μg/ml final concentration). A western blot analysis was then performed on the vaccine to verify the presence of the three proteins.

8. After visualization of the immunodominant proteins, the mixture was then precipitated with an equal volume of acetone and pelleted at 20,000 xg for 30 minutes in a refrigerated centrifuge.

9. The pellet was resuspended in sterile distilled water at ~2.0 mg/ml protein concentration.

10. The mixture was dialyzed using a membrane cut off point of 10,000 MW.

11. The sterility of the vaccine was confirmed by culturing 100 μl of the mixture on blood

agar and Sabouraud dextrose broth.

12. The vaccine was stored at 4°C until use.

EXAMPLE 2

One major drawback in evaluating the *P. insidiosum*-vaccine is the lack of an animal model. The only animal in which the disease can be successfully reproduced is the rabbit (*Orcytologous cuniculus*). But, no systematic studies have been conducted to evaluate its effectiveness as an experimental model. Evaluations of the *P. insidiosum*-vaccine has been carried out only in horses with the disease. The diagnosis of pythiosis in the treated equines was verified either by serology and/or culture, and by histopathology or all. Based on the fact that neither Miller's nor Mendoza's original vaccines cured infected horses after 60 days or more of infection, seven horses were selected with chronic pythiosis (>60 days of having the disease, some of them with more than 100 days after infection) and three with acute pythiosis (<60 days of having the disease), to conduct a vaccination trial with the vaccine containing the three proteins prepared as in Example 1.

The results indicated that the presence of these three immunodominant proteins remarkably enhanced Mendoza vaccine's curative properties. Of the seven vaccinated horses with chronic pythiosis four were cured, two did not respond, and one initially responded but died later. All of the cured horses developed a mild inflammatory reaction at their vaccination sites. However, the three horses that did not respond to the vaccinations did not develop such a reaction. Those horses had had their infections for more than 100 days and were considered to be anergic. This vaccine also cured all of the early cases of pythiosis.

The results of this experiment suggest that: 1) the presence of the three immunodominant proteins directly enhanced the curative properties of Mendoza's original vaccine which always failed in chronic cases

(>60 days) (Mendoza, L., et al., Mycopathologia **119**:89-95 (1992)), 2) these proteins are directly involved with the immunotherapeutic properties of Mendoza's vaccine, and 3) these proteins play a role in the immunology of *P. insidiosum* infection.

The findings also confirmed that the response to *P. insidiosum* vaccination is directly related to the immune status of the infected horse. Although the modified vaccine's main attribute is its ability to cure chronic equine pythiosis cases, it retained all of the properties of Mendoza's original vaccine. These include, the production of a mild inflammatory reaction at the site of vaccination in cured but not in unresponsive equines and 100% cure in early cases. The rate of cure using Mendoza's original vaccine was 48%. After addition of the 32K, 30K and 28K proteins, the rate of cure increased to 70%. The enhancement of its curative properties was directly related to the addition of the three prominent proteins to the original vaccine.

EXAMPLE 3

The genes that encode the three major proteins discussed in Example 2 can be cloned to dissect, at a molecular level, the components behind its protective and curative properties. The genes can be used to express the proteins in an expression vector in *E. coli* and combined to provide the improved vaccine.

EXAMPLE 4

This Example shows the use of the *Pythium insidiosum* vaccine (PIV) of Example 1 to successfully treat a Thai boy with a life threatening pythiosis insidiosi arteritis.

Methods

A 14 year-old boy presented with a history of progressive headache, mandibular soft tissue swelling, and facial nerve palsy. A computerized tomography scan of the head and neck showed abscesses in the bilateral retromolar fossa and in both ears. A non-sporulating

fungus-like organism was isolated in pure culture after surgical drainage of the abscesses. The organism was later identified as *Pythium insidiosum*. Despite treatment with amphotericin B, iodides, ketoconazole, and surgery, the infection progressed. A magnetic resonance imaging (MRI) and magnetic resonance analysis (MRA) of the neck revealed regional lymph node enlargement, stenosis and aneurysm in the external carotid artery. Surgical removal of the aneurysm was performed and *Pythium insidiosum* hyphae were histopathologically observed in the biopsied tissue. A MRA performed later showed stenosis of the internal carotid artery indicating that *Pythium insidiosum* had invaded this artery as well.

Based on the success of the improved vaccine (PIV) in animals with pythiosis insidiosi, vaccination was recommended as a last resort treatment. One hundred microliters of the PIV (2 mg/ml) was subcutaneously injected in the patient's left shoulder and 14 days later the same dose was repeated.

Results

Twenty-four hours post vaccination, a wheal and flare reaction had developed at the vaccination site (11 and 8 cm in diameter first and second vaccination, respectively). No other side effects occurred except for itching of the injection site. A second vaccination was performed two weeks later. Four weeks after the first vaccination the patient's headache had disappeared, his facial and left tongue swellings had dramatically diminished, the enlarged cervical lymph node had reduced in size, and the proximal left internal carotid artery stenosis had significantly improved. One year after the first vaccination the boy was considered clinically cured.

Conclusions

The dramatic events leading to the cure in this case, indicate that the use of PIV for the

immunotherapy of humans with pythiosis insidiosi should be considered in cases that do not respond well to the available chemotherapy.

In particular, a 14 year-old boy was admitted to the Ramathibodi Hospital, Bangkok, Thailand, with a history of 10 days of progressive headache. The illness had begun 16 days before admission in November 1995. Previous to the symptoms, he had developed a small skin injury on the posterior portion of his neck while swimming in a flooded area near a rice field. Four days after the skin injury, he developed three acne-like nodules at the injured site. He then was admitted to a local hospital with a severe headache and soft tissue swelling at the occiput. The swollen mass returned to normal after two days of dexamethazone treatment. The patient, however, continued to have severe headaches and developed a left facial nerve palsy before admission to the Ramathibodi Hospital.

The boy had a history of post splenectomy β-thalassemia hemoglobin E disease of four years duration. He had received at least three blood transfusions per year after his operation. Headache, bilateral facial nerve palsy, and progressively extensive facial cellulitis were recorded on admission. Empirical antibiotic treatment with cefotaxime 100 mg/kg/day and chloramphenicol 75 mg/kg/day were prescribed without success. A computerized tomography (CT) scan of the head and neck showed diffuse cellulitis. Abscesses in the bilateral retromolar fossa and in both ears were also observed. Pain and headache were relieved and the soft tissue swelling subsided after surgical drainage of the abscesses. A non-sporulating fungus-like organism was isolated in pure culture from tissue taken from the left and the right pinna. Because of the possibility of a fungal infection amphotericin B 0.5 mg/kg/day increasing to 1 mg/kg/day was administered. The isolate was later identified as *Pythium insidiosum*.

Although the abscess and cellulitis subsided, one week later, however, the pain and headache reappeared. Swelling of the left side of his tongue was also noticed. Saturated potassium iodide (1 g/ml) 3 ml/day that was increased gradually to 9 ml was prescribed. Despite this treatment, no clinical improvement was observed. Magnetic resonance images (MRI) of the head and neck demonstrated soft tissue involvement and regional lymph node enlargement. Surgical exploration of the left parapharynx and masseteric space was performed. During surgical exploration, the left abnormal cervical lymph nodes and the abnormal left great auricular nerve were removed. Histopathologically, the material showed follicular hyperplasia with sinus histiocytosis and granulomatous inflammation and aseptate hyphal elements of *Pythium insidiosum*. After failure with amphotericin B and iodides, chemotherapy with 300 mg/day of ketoconazole was initiated. Granulocyte macrophage colony stimulating factor (GM-CSF) was given 5 days immediately post surgical exploration.

The headache and swollen tongue improved after surgical intervention. Although treatment with ketoconazole and iodides continued, pain and headache reappeared three weeks later. A CT angiogram revealed an aneurysm in the left external carotid artery 1.0 cm above the bifurcation and stenosis with irregular walls of the internal carotid artery. A third surgical intervention was performed on February 1, 1996 to remove the aneurysm. The excised tissue was oval in shape 2.5 - 4 cm in diameter with necrotic-like material within its lumen. Histopathologically, eosinophils, marophages, CD3 positive T-cells, plasma cells, and hyphal elements of *Pythium insidiosum* were observed within the lumen and the vessel's wall. Pain and headache disappeared immediately after the surgical intervention. Five weeks after surgery, headache and

swelling tissue returned. A MRI and a MRA of the neck revealed the persistence of cervical and paracervical lymph node enlargement and persistent stenosis of the left internal carotid artery. These findings suggested that *Pythium insidiosum* had invaded that artery as well. Surgical removal of the left internal carotid artery was not recommended. Since amphotericin B, ketoconazole, iodides, surgery, and two courses of GM-CSF alone were ineffective in controlling the infection, *Pythium insidiosum* vaccine (PIV) was suggested as a last resort treatment.

Vaccine administration

A dose of 100μl of the 2 mg/ml PIV had been utilized to vaccinate horses with the disease. In successfully treated horses, an inflammatory reaction always developed at the site of vaccination. This inflammatory response indicated not only that the host's immune system was functioning, but it also predicted that the equine probably would be cured by the vaccine. Anergic horses with proven pythiosis insidiosi never developed such a reaction to the vaccine and did not respond to the immunotherapy (Mendoza, L., et al., Mycopathologia **94**:123-129 (1986); Newton, J. C., et al., Equine pythiosis: An overview of immunotherapy. Compendium **15**:491-493 (1993); and Miller, R. I., et al., J. Am Vet Med Assoc **182**:1227-1229 (1983)).

To avoid an excessive immunoresponse in the young boy with *Pythium insidiosum* arteritis, several dilutions of the original PIV were tested before the trial started. One hundred μl of each PIV dilution (1:100 to 1:100,000) were injected as a skin test on his right forearm. A mild inflammatory reaction was observed only with the 1:100 dilution of the PIV. Thus, the undiluted batch of PIV was selected. One hundred μl of the PIV was subcutaneously injected in the patient's left shoulder.

RESULT

Clinical course

Twenty hours after vaccination, a wheal and flare reaction had developed at the injection site. Forty-eight hours post vaccination, the wheal reaction attained its maximum size of 11 cm in diameter. No other side effects occurred except for itching at the vaccination site. The skin reaction disappeared 10 days post vaccination. Fourteen days after the first dose, the facial and tongue swelling had diminished. The same day a second vaccination was performed on the patient's right shoulder. Forty-eight hours later the wheal reaction at the vaccination had attained a diameter of eight centimeters. Two weeks after the second vaccination the patient's headache had disappeared, his facial and left tongue swelling were dramatically diminished, and the enlarged cervical lymph node had reduced in size. For the first time since his admission the patient's weight had increased by 4.0 kg four weeks post vaccination. The boy was considered clinically cured one year after the first vaccination.

MRI and MRA Findings

A MRI performed 6 weeks after the first vaccination, showed a decrease in the thickening of the soft tissue and less soft tissue enhancement of the left side of his tongue. A MRA of the neck released significant improvement of the stenosis of the proximal left internal carotid artery. The MRI and MRA twelve months post vaccination showed no infiltrations in the soft tissue and a normal left internal carotid artery.

Serology

A serum sample collected during the initial weeks post admission gave a negative results in an ID for pythiosis. Although the ID test in equine pythiosis is a reliable test some negative results have been reported in humans and dogs with proven pythiosis (Chetchotisakd, P., et al., J. Med Assoc Thailand

75:248-254 (1992); and Wanachiwanawin, W., et al., Trans Royal Soc Trop Med Hyg **87**:296-298 (1993)). When this serum was tested, before vaccination, in a new *Pythium insidiosum*-ELISA, positive titers of 1:6,400 were recorded. To monitor the vaccination's progress, sera collected one, two, six and twelve months post vaccination were also evaluated with the ELISA. A decrease in titers from 1:6,400 to 1:800 after 6 months post vaccination indicated that *Pythium insidiosum* may have been eliminated from the infected tissues, a finding that substantiated the clinical data. The antibody titer against *Pythium insidiosum* continued to decrease. However, low titers may persist for years as has been previously reported in equines cured by immunotherapy (Mendoza, L., et al., J. Clin Microbiol **30**:2980-2983 (1992)).

The response of the patient to PIV vaccine was remarkable. Besides the wheal and flare reaction at the site of vaccination no deleterious side effects developed. Within four weeks after immunotherapy his headaches had disappeared, tissue swelling decreased, and he gained 4.0 Kg in weight. Although the full strength vaccine was used (2 mg/ml) the patient tolerated PIV very well. The success obtained with the immunotherapy in this particular case suggests that PIV may be used as an alternative therapy for human pythiosis insidiosi. This finding is of importance because the available antifungal drugs have little effect on this emerging pathogen. This is the first human pythiosis insidiosi arteritis case treated and cured by the immunotherapeutic PIV.

Traditionally, vaccines have been used only for prophylactic purposes. The use of vaccines for the treatment of diseases, even though an old idea, has only recently received attention (Cohen, J., Science **264**:503-505 (1994)). The long-held medical dogma that vaccines are only for prevention has been challenged by

scientists working toward the development of immunotherapeutic vaccines against viruses (Burke, D. S., Vaccine **11**:883-890 (1993)), parasites (Convit, J., et al., Trans Royal Soc Trop Med Hyg. **87**:444-448 (1993)), bacteria (Standford, J. L., Trop Geograp Med **46**:93-107 (1994)), fungal (Gudding, R., et al., Can Vet J **36**:302-306 (1995)), and parafungal pathogens (Mendoza, L., et al., Mycopathologia **119**:89-95 (1992)). Despite impressive data originated by PIV and other curative vaccines, however, strong skepticism exists against the use of therapeutic vaccines as weapons for the treatment of infectious diseases. The skeptics have argued that when a host is invaded by an organism its immune system will mount an immune response that eventually will eliminate the invader. If the immune system fails, the use of drugs is the only avenue to pursue in efforts to save a patient's life. However, the findings generated by PIV and other therapeutic vaccines have indicated that a new line of research is necessary to investigate the mechanism by which these vaccines elicit an immunological reaction that kills the pathogens in infected tissues.

The mechanisms underlying the response to PIV are not well understood. However, based on histopathological and immunological studies in cured equines, it was found that the cellular immune response plays a major role in the clearance of *Pythium insidiosum* from infected tissues (Mendoza, L., et al., Mycopathologia **94**:123-129 (1986); Miller, R. I., Aust Vet J **67**:377-382 (1981); Newton, J. C., et al., Equine pythiosis: An overview of immunotherapy. Compendium **15**:491-493 (1993); and Mendoza, L., et al., Mycopathologia **119**:89-95 (1992)). These studies have shown that, after successful immunotherapy, the eosinophilic inflammatory reaction, typical of this disease, gradually changed to a mononuclear immunoresponse. Numerous macrophages, lymphocytes

(cytotoxic), and plasma cells had replaced the eosinophilic granuloma. Surprisingly, the mononuclear cells surrounded and killed *P. insidiosum's* hyphae, eliminating the pathogen from the affected tissues. This observation has been corroborated by the failure to recover *P. insidiosum* from the tissue of equines cured by immunotherapy (Newton, J. C., et al., Equine pythiosis: An overview of immunotherapy. Compendium **15**:491-493 (1993) and Mendoza, L., et al., Mycopathologia **119**:89-95 (1992)). Based on the PIV data accumulated in the past 15 years in equine pythiosis, it is strongly believed that the *P. insidiosum* vaccine displays to the host's immune system epitopes that are not well presented during natural infection. This scenario is possible since *Pythium insidiosum's* hyphae are always sequestered inside eosinophilic granulomas. Thus, *Pythium insidiosum* is probably using the degranulated eosinophils to hide important epitopes from the host's immune system.

It is intended that the foregoing description be only illustrative of the present invention and that the present invention be limited only by the hereinafter appended claims.

I CLAIM:

-1-

An injectable vaccine for treatment of Pythiosis which comprises in a sterile aqueous solution an admixture of:

(a) intracellular proteins separated from disrupted cells of *Pythium insidiosum*; and

(b) extracellular proteins from a supernatant from growing the cells of the *Pythium insidiosum*.

-2-

The vaccine of Claim 1 wherein the proteins have been provided by (1) growing cells of the *Pythium insidiosum* in a culture medium, then killing the cells, then separating the killed cells from the culture medium so as to produce a first supernatant and then disrupting the cells in water to provide the intracellular proteins in a second supernatant which are separated and (2) separating the extracellular proteins from the first supernatant.

-3-

The vaccine of Claim 2 wherein the cells have been disrupted by sonication.

-4-

The vaccine of Claim 1 wherein the *Pythium insidiosum* is deposited as ATCC 58643.

-5-

The vaccine of Claims 2 or 3 wherein the culture medium is Sabouraud dextrose broth.

-6-

The vaccine of Claim 2 wherein the cells are killed with thimersol.

-7-

The vaccine of Claim 2 wherein the disrupted cells are separated from the culture medium by centrifugation.

-8-

The vaccine of Claim 2 wherein the proteins have been separated by being precipitated together using acetone and then the precipitate is then dispersed in sterile distilled water, then dialyzed to remove low molecular weight components less than 10,000 MW to provide the vaccine.

-20-

-9-

A method for providing an injectable vaccine for treatment of Pythiosis which comprises:

(a) growing cells of *Pythium insidiosum* in a culture medium;

(b) separating the cells from a first supernatant of the culture medium which contains extracellular proteins;

(c) killing the cells;

(d) disrupting the cells in sterile water;

(e) separating the disrupted cells from the water to produce a second supernatant containing intracellular proteins;

(f) mixing the first supernatant of step (b) with the second supernatant of step (e);

(g) separating the combined proteins from the mixture of step (f);

(h) mixing the separated proteins in sterile distilled water; and

(i) dialyzing the mixture of step (h) to remove low molecular weight components less than 10,000 MW to produce the vaccine.

-10-

The method of Claim 9 wherein the cells are disrupted by sonication.

-11-

The method of Claim 9 wherein the *Pythium insidiosum* is deposited as ATCC 58643.

-12-

The method of any one of Claims 9, 10 or 11 wherein the culture medium is Sabouraud dextrose broth.

-13-

The method of Claim 9 wherein the cells are killed with thimersol.

-14-

The method of Claim 9 wherein the disrupted cells are separated from the water in step (e) by centrifugation.

-15-

The method of Claim 9 wherein the separated proteins are separated in step (g) by being precipitated together using acetone from the first and second supernatants combined together.

-16-

A method for treatment of Pythiosis in human patients having the disease which comprises:

(a) providing a vaccine containing separated proteins of *Pythium insidiosum* in a sterile aqueous solution; and

(b) vaccinating the patient with the vaccine.

-22-

-17-

The method of Claim 16 wherein the vaccination is subcutaneous.

-18-

A method for the treatment of Pythiosis in a mammal having the disease which comprises:

(a) providing an injectable vaccine which comprises in a sterile aqueous solution in admixture:

(1) an intracellular proteins separated from disrupted cells of *Pythium insidiosum;* and

(2) extracellular proteins from a supernatant from growing the cells of the *Pythium insidiosum;* and

(b) vaccinating the mammal with the vaccine.

-19-

The method of Claim 18 wherein in the proteins have been provided by growing cells of the *Pythium insidiosum* in a culture medium, then killing the cells, then separating the killed cells from the culture medium to produce a first supernatant and then disrupting the cells in water to provide the intracellular proteins in a second supernatant which have separated and (2) separating the extracellular proteins from the first supernatant.

-20-

The method of Claim 18 wherein the cells have been disrupted by sonication.

-23-

-21-

The method of Claim 18 wherein the *Pythium insidiosum* is deposited as ATCC 58643.

-22-

The method of any one of Claims 19, 20 or 21 wherein the culture medium is Sabouraud dextrose broth.

-23-

The method of Claim 19 wherein the cells are killed with thimersol.

-24-

The method of Claim 19 wherein the disrupted cells are separated from the culture medium for the cells by centrifugation.

-25-

The method of Claim 19 wherein the separated proteins have been precipitated together from the first and second supernatants combined together using acetone and then dispensed in sterile distilled water to provide the vaccine.

ABSTRACT IF THE DISCLOSURE

A method and vaccine for treatment of pythiosis in humans and animals is described. In particular a vaccine comprising a mixture of extracellular and intracellular proteins is described. The vaccine enables cures of chronic pythiosis in some patients.

Attorney's Docket No. MSU 4.1-356 ***PATENT***

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION OR C-I-P)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type:

(check one applicable item below)

- ☒ original.
- ☐ design.
- ☐ supplemental.

NOTE: If the declaration is for an International Application being filed as a divisional, continuation or continuation-in-part application, do not check next item; check appropriate one of last three items.

- ☐ national stage of PCT.

NOTE: If one of the following 3 items apply, then complete and also attach ADDED PAGES FOR DIVISIONAL, CONTINUATION OR C-I-P.

- ☐ divisional.
- ☐ continuation.
- ☐ continuation-in-part (C-I-P).

INVENTORSHIP IDENTIFICATION

WARNING: *If the inventors are each not the inventors of all the claims, an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.*

My residence, post office address and citizenship are as stated below, next to my name. I believe that I am the original, first and sole inventor *(if only one name is listed below)* or an original, first and joint inventor *(if plural names are listed below)* of the subject matter that is claimed, and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

METHOD AND VACCINE FOR TREATMENT OF PYTHIOSIS INSIDIOSI IN HUMANS AND LOWER ANIMALS

SPECIFICATION IDENTIFICATION

the specification of which:

(complete (a), (b) or (c))

(a) ☒ is attached hereto.

NOTE: "The following combinations of information supplied in an oath or declaration filed on the application filing date with a specification are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 CFR 1 63:

"(1) name of inventor(s), and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration on filing;

"(2) name of inventor(s), and attorney docket number which was on the specification as filed; or

"(3) name of inventor(s), and title which was on the specification as filed."

Notice of July 13, 1995 (1177 O.G. 60).

(b) ☐ was filed on _____, as ☐ Serial No. 0 /_____ or ☐ _____ and was amended on _____ *(if applicable).*

NOTE: Amendments filed after the original papers are deposited with the PTO that contain new matter are not accorded a filing date by being referred to in the declaration. Accordingly, the amendments involved are those filed with the application papers or, in the case of a supplemental declaration, are those amendments claiming matter not encompassed in the original statement of invention or claims. See 37 CFR 1.67.

NOTE: "The following combinations of information supplied in an oath or declaration filed after the filing date are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 CFR 1.63:

"(1) name of inventor(s), and application number (consisting of the series code and the serial number; e.g.,08/123,456);

"(2) name of inventor(s), serial number and filing date;

"(3) name of inventor(s) and attorney docket number which was on the specification as filed;

"(4) name of inventor(s), title which was on the specification as filed and filing date;

"(5) name of inventor(s), title which was on the specification as filed and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration; or

"(6) name of inventor(s), title which was on the specification as filed and accompanied by a cover letter accurately identifying the application for which it was intended by either the application number (consisting of the series code and the serial number; e.g.,08/123,456), or serial number and filing date. Absent any statement(s) to the contrary, it will be presumed that the application filed in the PTO is the application which the inventor(s) executed by signing the oath or declaration "

Notice of July 13, 1995 (1177 O G. 60)

(c) ☐ was described and claimed in PCT International Application No. _____, filed on _____ and as amended under PCT Article 19 on _____ *(if any).*

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, § 1.56,

(also check the following items, if desired)

☒ and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable Examiner would consider it important in deciding whether to allow the application to issue as a patent, and

☒ in compliance with this duty, there is attached an information disclosure statement, in accordance with 37 CFR 1.98.

PRIORITY CLAIM (35 U.S.C. § 119(a)–(d))

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)–(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

(complete (d) or (e))

(d) ☒ no such applications have been filed.

(e) ☐ such applications have been filed as follows.

NOTE: Where item (c) is entered above and the International Application which designated the U.S. itself claimed priority check item (e), enter the details below and make the priority claim.

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. § 119(a)-(d)

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING (day, **month**, year)	PRIORITY CLAIMED UNDER 37 USC 119
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(34 U.S.C. § 119(e))

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

PROVISIONAL APPLICATION NUMBER **FILING DATE**

_____ /_____ _____

_____ /_____ _____

_____ /_____ _____

CLAIM FOR BENEFIT OF EARLIER US/PCT APPLICATION(S) UNDER 35 U.S.C. 120

☐ The claim for the benefit of any such applications are set forth in the attached ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN PART (C-I-P) APPLICATION.

ALL FOREIGN APPLICATION(S), *IF ANY*, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

_____

_____

_____

NOTE: If the application filed more than 12 months from the filing date of this application is a PCT filing forming the basis for this application entering the United States as (1) the national stage, or (2) a continuation, divisional, or continuation-in-part, then also complete ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR C-I-P APPLICATION for benefit of the prior U.S. or PCT application(s) under 35 U.S.C. § 120.

POWER OF ATTORNEY

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

(list name and registration number)

Ian C. McLeod Registration No. 20,931

(check the following item, if applicable)

☐ Attached, as part of this declaration and power of attorney, is the authorization of the above-named attorney(s) to accept and follow instructions from my representative(s).

SEND CORRESPONDENCE TO

Ian C. McLeod
2190 Commons Parkway
Okemos, Michigan 48864

DIRECT TELEPHONE CALLS TO:
(Name and telephone number)

Ian C. McLeod
(517) 347-4100

DECLARATION

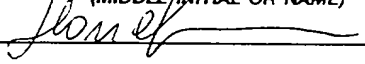
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

NOTE: Carefully indicate the family (or last) name, as it should appear on the filing receipt and all other documents.

Full name of sole or first inventor

Alberto (GIVEN NAME) L. (MIDDLE INITIAL OR NAME) Mendoza (FAMILY (OR LAST NAME))

Inventor's signature 

Date July 15, 1997 **Country of Citizenship** Costa Rica

Residence Haslett, Michigan

Post Office Address 1745 Nemoke Trail

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Full name of second joint inventor, if any

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Inventor's signature _____

Date _____ **Country of Citizenship** _____

Residence _____

Post Office Address _____

Full name of third joint inventor, if any

(GIVEN NAME) (MIDDLE INITIAL OR NAME) (FAMILY (OR LAST NAME))

Inventor's signature _____

Date _____ **Country of Citizenship** _____

Residence _____

Post Office Address _____

(check proper box(es) for any of the following added page(s) that form a part of this declaration)

☐ **Signature** for fourth and subsequent joint inventors. *Number of pages added* _____

* * *

☐ **Signature** by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. *Number of pages added* _____

* * *

☐ **Signature** for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR 1.47. *Number of pages added* _____

* * *

☐ Added page for **signature** by one joint inventor on behalf of deceased inventor(s) where legal representative cannot be appointed in time. (37 CFR 1.47)

* * *

☐ Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (C-I-P) application.

☐ Number of pages added _____

* * *

☐ Authorization of attorney(s) to accept and follow instructions from representative.

* * *

(if no further pages form a part of this Declaration, then end this Declaration with this page and check the following item)

☒ This declaration ends with this page.

United States Patent & Trademark Office

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